It may be necessary to illuminate an encoded bead with light to enable the encoded bead to be read. In one embodiment, the encoded beads are entrained in a fluid, and the encoded beads are imaged in a flow of the fluid. However, the present invention does not require that the beads be imaged in a fluid flow.

Preferably the encoded beads are analyzed by determining one or more portions of each image corresponding to a unique reporter, and then determining a signature of each reporter based on the image data. The reporters thus identified determine the identity and the sequence in which the sub units that form the compound attached to the encoded bead were added.

A predefined number of unique reporters should be detected on an encoded bead. The method can also include the step of disregarding all signals relating to an encoded bead if the plurality of signals for that encoded bead indicate that fewer reporters are associated with the encoded bead than were expected. This step can be facilitated by referring to an encoded bead legend that relates each unique reporter to a specific sub unit and a specific disposition of that sub unit in a sequence that forms a compound.

It should also be noted that encoded beads are usable to analyze DNA sequences, or for other DNA related research. In such an application, it is preferable not to collect data for encoded beads that have not experienced a binding event. Beads that have experienced a binding event are referred to herein as "positive" beads. In at least one embodiment of the present invention, only data from positive beads are analyzed.

Several different embodiments of imaging apparatus are usable to image and decode encoded beads. In general, the apparatus will include a collection lens to collect and focus light from an encoded bead in a desired direction, a dispersing component that receives the light from the collection lens and disperses the light into a plurality of light beams as a function of a plurality different discriminable characteristics of the light, such that the plurality of different discriminable characteristics are indicative of a plurality of different reporters. Also included is at least one pixelated detector; and an imaging lens that focusses each of the plurality of light beams on the at least one pixelated detector, thereby producing a respective image corresponding to each of the plurality of light beam. The pixelated detector provides an output signal for each respective image, such that each output signal may individually, or in combination with other output signals, indicate whether a different reporter is present on the encoded bead. A signal processor is coupled to the pixelated detector to receive its output signal and processes the output signal to decode the identity and sequence of the sub units that

form the compound attached to an encoded bead, or alternatively, to decode the bead to identify the compound using a cross reference table.

Preferably, in one exemplary application, the signal processor is adapted to generate sequence contigs from a plurality of decoded sequences representing a plurality of encoded beads. Such sequence contigs preferably are used to identify at least one of a genomic DNA sequence, a polymorphic allele, and an expressed gene.

The signal processor can be adapted to disregard all output signals relating to a reporter if signals from an identical reporter have already been analyzed. The signal processor can similarly be adapted to disregard all output signals for an encoded bead if the signals indicate that fewer than a predetermined number of reporters are associated with the encoded bead. The signal processor is preferably adapted to employ an encoded bead legend that relates each unique reporter to a specific sub unit and a specific disposition in a sequence of sub units that comprise the compound associated with the encoded bead. By employing such an encoded bead library, the signal processor can selectively disregard all output signals if it is determined that the encoded bead does not correspond to that encoded bead legend. Alternatively, the signal processor can be adapted to employ a cross reference table (such as the reporter legend of FIGURE 19) to relate bead identity to compound identity. Also, the signal processor can be controlled to only analyze signals relating to positive encoded beads.

In at least one embodiment, the encoded beads are entrained in a fluid and imaged while the fluid is flowing through the imaging system. The imaging system includes a bead reservoir containing encoded beads that have not been imaged, and fluid channels to control a flow of fluid. A light source is optionally included to illuminate the encoded bead to be imaged.

The dispersing component is preferably either a prism or a plurality of dichroic filters. If a prism is employed, the images will be convolved and the signal processor will need to deconvolve the images. One or more time delay and integration (TDI) detectors are also preferably included in the imaging system to detect the light from the beads. In one embodiment, a plurality of dichroic filters, imaging lenses, and detectors are included.

Still another aspect of the present invention relates to a method for simultaneously imaging a plurality of reporters disposed on substantially different portions of an encoded bead, to identify each unique reporter included on the encoded bead. The method includes the steps of receiving light from the encoded bead along a plurality of collection paths that are substantially spaced apart, such that light from the reporters disposed on the different portions of the encoded bead affect the light received

therefrom. The light is processed to identify each unique reporter included on the encoded bead.

A final aspect of the present invention is directed to a method for employing an oligo library encoded on beads for at least one of DNA sequencing, a polymorphism analysis, and an expression analysis. An imaging system capable of decoding a sequence of encoded beads conveyed in a flow of fluid is provided. Essentially, a complete encoded bead library of N-mer oligos is generated, and the library is used to perform either the DNA sequencing, the polymorphism analysis, or the expression analysis. Positive beads are imaged and decoded using imaging data produced by an imaging system such as described above.

When DNA sequencing is the analysis function that is selected, the genomic DNA component is amplified using primers for extended sequences of interest. When a polymorphism analysis function is selected, the genomic DNA component is amplified using primers for polymorphic regions of interest. Alternatively, when an expression analysis function is selected, the ribonucleic acid (RNA) component is amplified using primers for genes of interest.

Regardless of the specific analysis function that is selected, the amplified component is hybridized to the encoded bead library; and the imaging system is employed to identify oligo sequences of encoded beads hybridized as noted above. From the imaging data, sequence contigs are constructed from the oligo sequences identified by the imaging analysis to identify either a genomic DNA sequence, a polymorphic allele, or an expressed gene. In a preferred embodiment, the N-mer oligos comprise oligos having a length equal to ten.

Brief Description of the Drawing Figures

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same becomes better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 is an isometric view of an embodiment of an imaging system useful for imaging reporter beads, in which multiple legs are employed for the spectral decomposition and imaging to collect light signals from multiple perspective positions;

FIGURES 2A and 2C are isometric views of two related embodiments of an imaging system using a prism for spectral dispersion of light and usable for imaging reporter beads, wherein FIGURE 2C also includes a slit for spatial filtering of extraneous light;

FIGURE 2B is an schematic view of embodiment 2A showing bead traversal along a direction of motion in both object and image space;